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ORIGINAL ARTICLE

Nigella sativa and thymoquinone attenuate oxidative stress and cognitive impairment following cerebral hypoperfusion in rats

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Abstract

Nigella sativa, a plant widely used in traditional medicine, possesses anti-inflammatory, antioxidant and neuroprotective properties. In the present study, we investigated the effect of hydroalcoholic extract of *N. sativa* seeds (NSE) and its active constituent, thymoquinone (TQ), on learning and memory deficits, hippocampal acetylcholine esterase (AChE) activity, and markers of redox status, mainly lipid peroxidation and superoxide dismutase (SOD) activity following cerebral hypoperfusion in rats. Cerebral hypoperfusion was induced by permanent occlusion of bilateral common carotid arteries (2VO). Male Wistar rats were administered either a vehicle (sham group: 10 ml/kg/day, ip), NSE (100, 200, and 400 mg/kg/day, ip), TQ (10, 20, and 40 mg/kg/day, ip), or donepezil (5 mg/kg/day, ip) for 10 days (three days before and seven days after ligation). Spatial learning and memory deficits were investigated using the Morris water maze (MWM) task. 2VO produced significant learning and memory deficits as evidenced by increased latency time to reach the hidden platform, increased swimming time, and decreased time spent in the target quadrant in the probe trial in the MWM task. There was also a significant increase in the lipid peroxidation level and AChE activity, and a significant decrease in SOD activity in the hippocampal portion of hypoperfused rats, as compared with the sham group. Treatment with NSE (400 mg/kg/day; $p < 0.001$) and TQ (40 mg/kg/day; $p < 0.001$), as well as donepezil significantly prevented learning and memory impairments and alleviated changes in the hippocampal lipid peroxide level and SOD and AChE activities in this model. In conclusion, our data suggest that *N. sativa* and thymoquinone have a beneficial role in cerebrovascular insufficiency states and dementia.

Keywords *Nigella sativa* · Thymoquinone · Cerebral hypoperfusion · Learning and memory · Morris water maze (MWM)

Introduction

Vascular dementia (VaD), which is the second most common type of dementia in the elderly population after Alzheimer's disease (AD), is initiated by cerebrovascular disease with pathological features of infarction and cerebral arteriosclerosis (Gunstad et al. 2005; He et al. 2008; Liu et al. 2010; Royall 2002). A chronic decrease resulting in cerebral blood flow occurs during the onset of VaD and the accompanying cognitive deficits. Patients with vascular dementia usually experience learning and memory impairments (Jing et al. 2015). Between the numerous fundamental explanations for memory damage, cholinergic system dysfunction in the cortical and hippocampal regions has been recognized as one of the main reasons for these atypical performances (Wang et al. 2009). AD and other types of dementia can be treated using agents that either restore the level of neurotransmitter acetylcholine (ACh) by inhibiting acetylcholinesterase (AChE), such as donepezil, or preventing the excessive stimulation of the glutamate system such as memantine (Raina et al. 2008).

Permanent occlusion of bilateral common carotid arteries (two-vessel occlusion, 2VO) in rat is considered as an appropriate model to display chronic brain hypoperfusion condition, which is documented to be a key factor in Alzheimer's disease or/and vascular dementia (Yan and Ai 2017). Some of the main pathological features of 2VO rat model are including impaired learning and memory (Liu et al. 2005), oxidative

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stress (Saxena et al. 2015), and neuroinflammation (Moghaddasi et al. 2017). Moreover, the 2VO model in the rat can be used for evaluation of the neuroprotective strategies in the chronic cerebral hypoperfusion-induced vascular dementia (Farkas et al. 2007; Gupta et al. 2016).

Several medicinal herbs have recently been considered as therapeutic agents in the management of neurological disorders induced by cerebrovascular insufficiency (Asai et al. 2007; Dhanasekaran et al. 2007; Kim et al. 2007). *Nigella sativa* is an annual plant belonging to the Ranunculaceae family that is cultivated for its black seeds in different areas, especially in the Middle East. It is added to a variety of foods (e.g., curries, pastries, honey, breads, and cheeses) to give a slightly bitter, peppery taste (Ramadan 2007). *N. sativa* has conventionally been used to treat a diversity of chronic diseases such as hypertension, hypercholesterolemia, inflammation, asthma, bronchitis, cough, dizziness, fever, headache, and influenza (Ali and Blunden 2003). Furthermore, it is also used to alleviate skin diseases, for instance psoriasis and eczema (Ramadan 2007). Thymoquinone (TQ), the most bioactive compound present in the black seed, also has a variety of beneficial effects (Dehkordi and Kamkhah 2008; Norwood et al. 2005; Rchid et al. 2004). TQ has shown a protective role against ethanol-induced neuronal apoptosis in primary rat cortical neurons (Sedaghat et al. 2014; Ullah et al. 2012), and protects PC12 cells against cytotoxic agents via attenuation of oxidative stress (Mousavi et al. 2010; Sedaghat et al. 2014). TQ could also ameliorate neurodegeneration in the frontal cortex after chronic toluene exposure in rats (Kanter 2011). The potential ability of TQ to protect dopaminergic neurons in cell culture against MPP^+ (1-methyl-4-phenylpyridinium) and rotenone cytotoxicity has previously been reported (Radad et al. 2009; Sedaghat et al. 2014). In a rabbit model following 5 mg/kg (intravenous, IV) or 20 mg/kg (oral) TQ administration, the clearance rate was 7.19 and 12.30 mL/kg/min, the estimated volume of distribution at a steady state was 700.90 and 5109.46 mL/kg, and the elimination half-life ($t_{1/2}$) was 63.43 and 274.61 min, respectively. The protein binding and oral bioavailability of TQ were 99% and 58%, respectively (Alkharfy et al. 2015).

The aim of the present study was to determine the effect of *N. sativa* and its constituent, thymoquinone, on learning and memory impairments, AChE activity, and oxidative stress following cerebral hypoperfusion in rats.

Materials and methods

Preparation of the hydroalcoholic extract of *N. sativa* (NSE)

The seeds of *N. sativa* were obtained from the Medicinal Plants Division of Imam Reza Pharmacy and identified by a botanist in the Herbarium of the Ferdowsi University of Mashhad (No. 293–0303-1). After washing, the seeds were dried and then crushed to a powder by using an electric microniser. To obtain an extraction of the black seed, 100 g seeds were soaked in 500 mL of 70% ethanol and gently agitated at 40 °C for 72 h. The resulting extract was then concentrated under reduced pressure and kept at −20 °C until use (yielded 23%).

Chemicals

5, 5′-Dithiobis-2-nitrobenzoic acid (DTNB), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), tris (hydroxymethyl) aminomethane (Trizma base), sodium acetate, and glacial acetic acid were purchased from Merck (Darmstadt, Germany). Pyrogallol, methylthiazolyldiphenyl-tetrazolium bromide (MTT), phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO), bicinchoninic acid (BCA) protein assay kit, and acetylthiocholine iodide were purchased from Sigma (St. Louis, USA).

Animals

Male Wistar rats weighing 200–230 g were obtained from the Animal Facility of the Avicenna Research Institute of the Mashhad University of Medical Sciences. The animals were housed five per cage with a 12 h light/dark cycle at 21 ± 2 °C and they had free access to food and water, ad libitum. All animals were treated in accordance with the National Institutes of Health Guidance for the Care and Use of Laboratory Animals, and their use was approved by the Animal Ethics Committee of the Mashhad University of Medical Sciences (No. 88487).

Surgery

Chronic cerebral hypoperfusion was induced by bilateral occlusion of the common carotid arteries (2VO) adapted from Xu et al. with minor modifications (Hosseinzadeh et al. 2012). Briefly, rats were anesthetized with chloral hydrate (350 mg/kg, intraperitoneally) and the left and right common carotid arteries were exposed through a midline neck incision and double ligated with a 4–0 type surgical suture. During the surgery, the body temperature was monitored and maintained at 37.5 ± 0.5 °C by a heating lamp.

The rats were randomly divided into nine groups (8–10 rats per group): The sham-operated animals underwent the same surgical procedure as the other groups without 2VO and received 0.9% saline solution. The cerebral hypoperfused rats underwent permanent 2VO and received 0.9% saline solution (2VO group). The NSE and TQ treatment groups received either NSE (50, 100 and 250 mg/kg/day, ip) or TQ (5, 10, 25 mg/kg/day, ip), three days before and seven days after

2VO. The donepezil group was administrated donepezil (5 mg/kg/day, ip) for 10 days (three days before and seven days after ligation).

Assessment of learning and memory deficits

Seven days after surgery, the MWM task was used to evaluate spatial memory and learning performances (Hosseinzadeh et al. 2012). MWM is a black circular tank (35 cm deep, 136 cm diameter, and 60 cm high) and it was filled with water (22–24 °C) approximately 2 cm above the circular platform (10 cm diameter), which was located in the center of the southwest quadrant (target quadrant).

Seven-day training in the MWM was done (one block of four trials). Spatial learning and memory was assessed by measuring the traveled distance, escape latency, and swimming speed through a video tracking system same as described previously (Hosseinzadeh et al. 2012). The time spent in the target quadrant was also evaluated by performing the probe trial on day 8 with the removed platform. In the probe trial, animals were allowed to swim freely for 60 s.

Biochemical analysis

After the last session of the MWM task, the rats were decapitated and the brains were quickly removed and placed in ice-cold saline. The hippocampus portions were quickly dissected and placed on a petri dish chilled on crushed ice, and then weighed and homogenized (20%) in 0.1 M phosphate buffer (pH = 8.0) and used for biochemical measurements.

Thiobarbituric acid reactive species measurement

Hippocampal lipid peroxidation was assayed by its end product formation, malondialdehyde (MDA), that reacts with thiobarbituric acid (TBA) as a substrate to generate a pink colored compound which has peak absorbance at 535 nm (Buege and Aust 1978). In brief, 0.5 ml of the homogenate sample was combined with 1 ml of TCA-TBA-HCl mixture (15% TCA, 0.67% TBA, and 0.25 N HCl) and placed for 45 min in a boiling water bath. After cooling, this combination was centrifuged for 10 min at 3000 rpm for 10 min after collecting the supernatant, and its absorbance at 535 nm was read against the blank. The amount of MDA production was calculated and then reported as nmol/g tissue by means of a molar absorption coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Sadeghnia et al. 2013).

Assessment of superoxide dismutase (SOD) activity

SOD activity was measured according to the method described by Madesh and Balasubramanium (Madesh and Balasubramanian 1998). Briefly, the reaction mixture containing 10 μL homogenate, 75 μl pyrogallol (100 μM), 30 μl MTT (1.25 μM), and 0.65 ml PBS (pH 7.4), which was kept at room temperature for five minutes and then the reaction was finished by adding 0.75 ml DMSO. The absorbance of this mixture was read at 570 nm against distilled water. The activity of the enzyme has been expressed as unit/mg protein. One unit of enzymatic activity is the μg of protein required to inhibit MTT reduction by 50%. The protein content was measured using a BCA kit.

Assay of AChE activity

The Ellman et al. method, as previously described, was used to determination the AChE activity in the hippocampus (Ellman et al. 1961). Briefly, 2.6 mL of phosphate buffer (pH 8.0, 0.1 M) and 0.1 mL of 10 mM DTNB was added to 0.4 mL of the homogenate sample and the absorbance was measured at 412 nm. After that, 0.02 mL of 75 mM acetylthiocholine iodide was added to the mixture. Changes in absorbance were recorded at 412 nm for five minutes. AChE activity was calculated using the following formula and expressed as μmol/min/mg protein (Ellman et al. 1961).

$$\text{AChE activity} = 5.74 \times 10^{-4}\ \Delta A/C$$

where ΔA is the change in absorbance per min and C is the original concentration of tissue (mg/ml).

Statistical analysis

Data are expressed as mean ± SEM. The escape latency, traveled distance, and swimming speed were analyzed by the two-way analysis of variance (ANOVA) followed by the Bonferroni's post hoc test. The retention probe memory test and biochemical data were analyzed by the one-way ANOVA followed by the Tukey's post hoc test. The p values less than 0.05 were considered statistically significant.

Results

NSE and TQ improved learning and memory deficits induced by 2VO

As shown in Fig. 1a-b, 2VO animals showed significantly higher escape latency than the sham-operated rats from day 2 ($p < 0.05$) to day 7 ($p < 0.001$). NSE significantly improved learning performances as compared with the 2VO group, in a dose-dependent manner (Fig. 1a). The mean escape latency for NSE 100, 200, and 400 mg/kg groups at day 7 were 40.4 ± 3.1, 21.6 ± 2.4 and 7.8 ± 1.3 s respectively, whereas it was 59.7 ± 4.8 s for 2VO rats ($p < 0.01$). TQ also significantly decreased the mean latency time dose-dependently, as compared with the 2VO group (Fig. 1b). The latency time for TQ

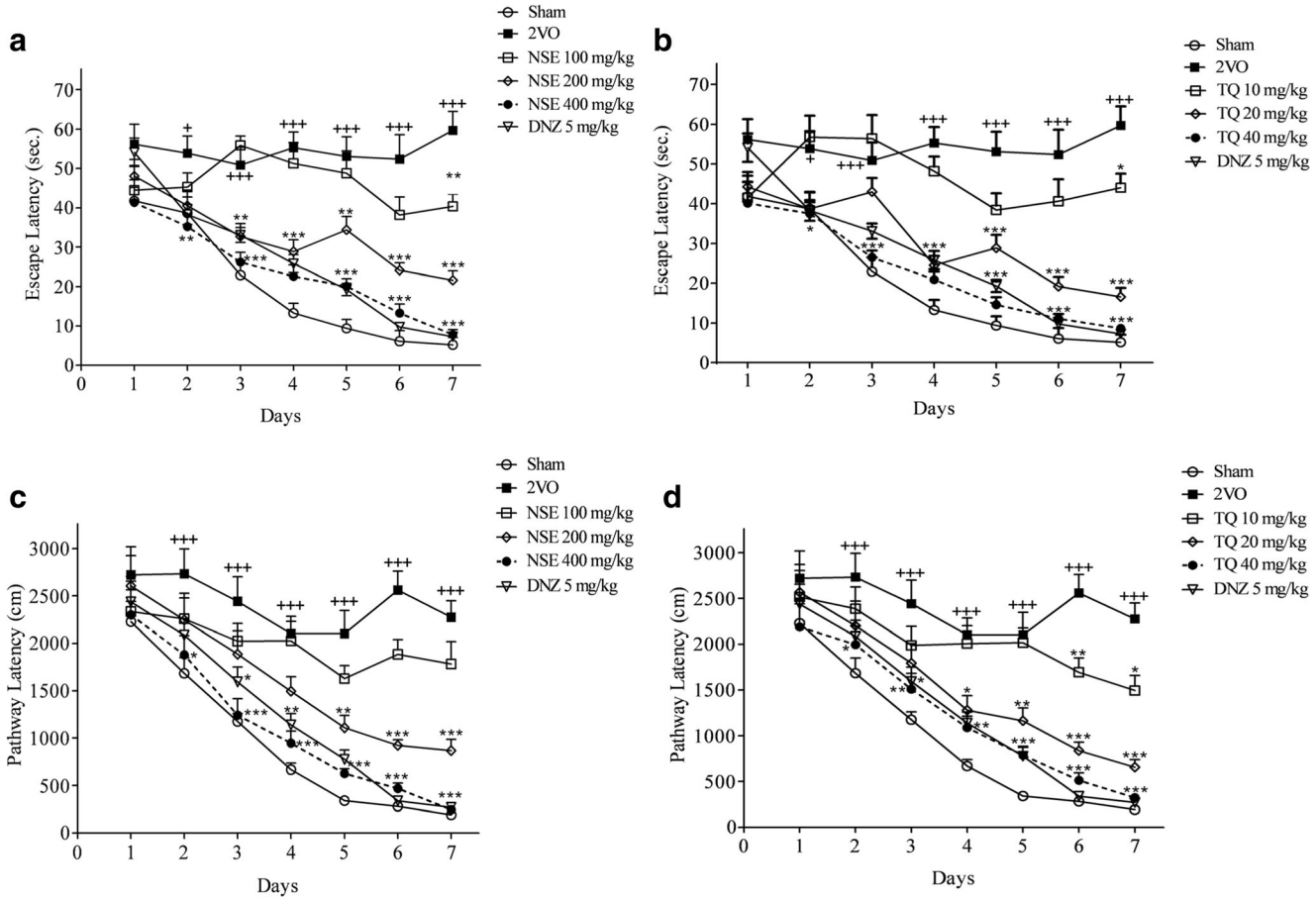

Fig. 1 Effects of *N. sativa* hydroalcoholic extract (NSE) and thymoquinine (TQ) on escape latency (**a**, **b**) and pathway latency (**c**, **d**) in Morris water maze (MWM) task. Values are means ± SEM ($n = 10$). $^{*}p < 0.05$, $^{**}p < 0.05$ and $^{***}p < 0.001$ vs. two vessels occluded (2VO) rats, $^{+++}p < 0.001$ vs. sham group

10, 20, and 40 mg/kg groups at day 7 were 44 ± 3.6, 16.6 ± 2.2, and 8.7 ± 0.9 s, respectively ($p < 0.05$ as compared with hypoperfused animals).

According to Fig. 1c-d, the mean distance travelled by untreated 2VO rats was significantly longer than the sham, NSE (200 and 400 mg/kg), TQ (10, 20 and 40 mg/kg), or donepezil-treated groups. Statistical analysis also revealed that the effect of NSE and TQ was dose dependent.

The swimming time in the target quadrant was used to assess spatial memory performance. Figure 2a-b shows that the sham-operated animals, NSE (400 mg/kg), and TQ (40 mg/kg)-treated groups swam significantly longer in the target quadrant than the 2VO group. The time traveled in the target quadrant in the sham-operated group was 46.8 ± 5.3 s, in comparison with the 2VO group (22.6 ± 3.2 s, $p < 0.01$). In the NSE (400 mg/kg) group, it was augmented to 43.7 ± 4.6 s ($p < 0.05$). Also, TQ (40 mg/kg)-treated cerebral hypoperfused rats spent more time in the target quadrants (47.1 ± 5.3 s, $p < 0.01$).

As illustrated in Fig. 3a-b, no significant differences were seen in the swimming speed between the treatment groups during the experiment, which revealed no sensorimotor effect.

NSE and TQ alleviated hippocampal oxidative stress in 2VO rats

As shown in Fig. 4a-b, NSE and TQ decreased MDA concentration in a dose-dependent manner. The results of the MDA change showed that the MDA level in the 2VO animal group was significantly higher than the sham-operated animals (233.6 ± 21.2 vs. 144.7 ± 9.8 nmol/g tissue, $p < 0.001$; Fig. 4a-b). Treatment with the NSE (200 and 400 mg/kg) and TQ (20 and 40 mg/kg) resulted in a significant decrease in the lipid peroxidation as illustrated by a reduction in MDA levels, in comparison with the 2VO group (Fig. 4a-b).

NSE and TQ also at high concentrations significantly restored compromised SOD activity following cerebral hypoperfusion. Following 2VO induction, the SOD activity was significantly diminished in the brains of the rats, as compared to the sham animals (1.6 ± 0.2 vs 4.2 ± 0.7 IU/min/mg protein; $p < 0.01$). Treatment of cerebral hypoperfused rats with NSE 200 (3.8 ± 0.3 IU/min/mg protein; $p < 0.05$), NSE 400 mg/kg (4.4 ± 0.6 IU/min/mg protein; $p < 0.01$), and TQ 40 mg/kg (4 ± 0.3 IU/min/mg protein; $p < 0.01$) significantly improved the SOD activity to nearly sham value (Fig. 5a-b).

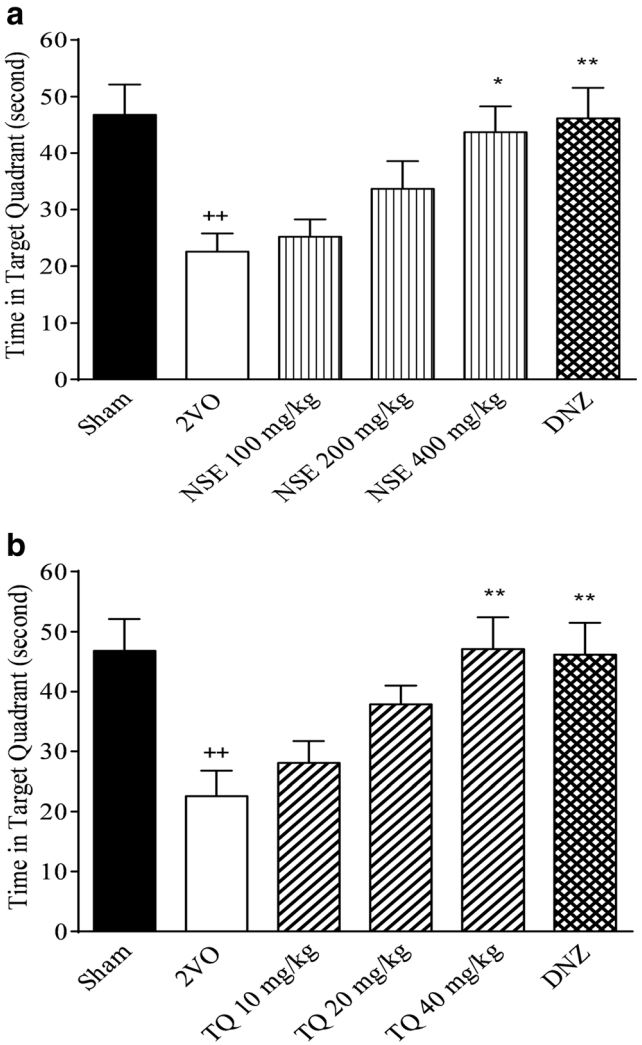

Fig. 2 Effects of *N. sativa* hydroalcoholic extract (NSE) (**a**) and thymoquinine (TQ) (**b**) on time spent in target quadrant in Morris water maze (MWM) task. Values are means ± SEM (n = 10). $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. two vessels occluded (2VO) rats, $^{++}p < 0.01$ vs. sham group

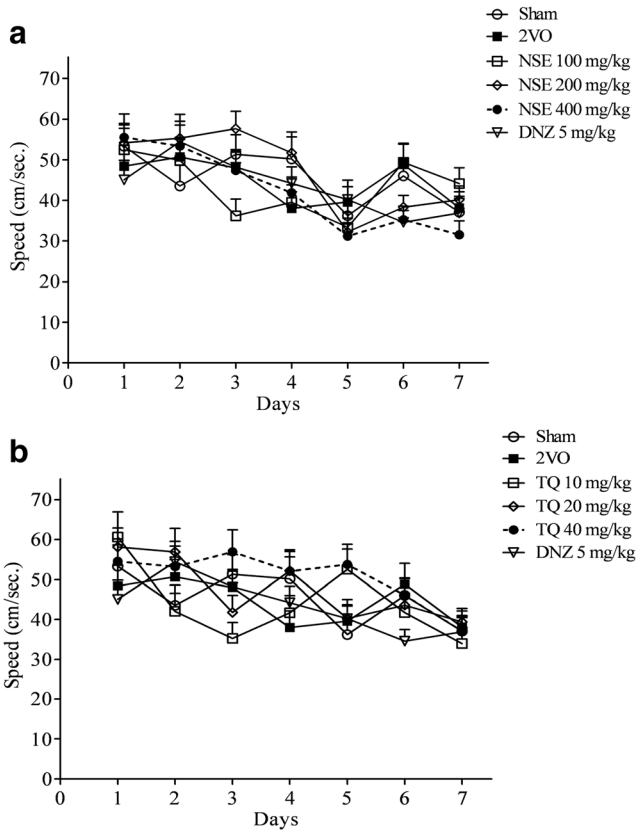

Fig. 3 Effects of *N. sativa* hydroalcoholic extract (NSE) (**a**) and thymoquinine (TQ) (**b**) on swimming speed in Morris water maze (MWM) task. Values are means ± SEM (n = 10). No significant differences were seen between sham-, two vessels occluded (2VO)-, NSE treated or TQ treated rats

NSE and TQ increased AChE activity in a dose-dependent manner

Figure 6a-b shows that there was a significant increase ($p < 0.001$) in the AChE activity in the hippocampus of 2VO rats (0.95 ± 0.03 μmol/min/mg protein), as compared to the sham-operated rats (0.44 ± 0.02 μmol/min/mg protein). Administration of NSE 200 mg/kg (0.62 ± 0.02 μmol/min/mg protein; $p < 0.001$) and NSE 400 mg/kg (0.45 ± 0.05 μmol/min/mg protein; $p < 0.001$) reduced the AChE activity when compared to the 2VO group. Treatment of hypoperfused animals with TQ 20 mg/kg (0.71 ± 0.05 μmol/min/mg protein; $p < 0.001$) and TQ 40 mg/kg (0.64 ± 0.01 μmol/min/mg protein; $p < 0.001$) also decreased AChE activity in comparison with the 2VO group rats.

Discussion

To the best of our knowledge, our study is the first to evaluate the effects of NSE and TQ on impaired cognition in the rat model of 2VO (using the MWM task) and we were able to demonstrated that treatment of 2VO rats with NSE or TQ could ameliorate the cognitive impairments produced by chronic hypoperfusion with concurrent improvements in hippocampal oxidative stress and cholinergic deficits.

In this study, the bilateral occlusion of the carotid arteries of Wistar rats was used to produce cerebral hypoperfusion, which resulted in learning and memory acquisition impairments. 2VO also significantly increased the hippocampal lipid peroxidation and AchE activity, along with a significant reduction in SOD activity, which is consistent with previous studies (Hosseinzadeh et al. 2012; Ma et al. 2017; Vattananupon et al. 2017; Zhang et al. 2018). Chronic cerebral hypoperfusion, a pathological reason for the initiation of VaD, is commonly modeled by the permanent ligation of bilateral common carotid arteries in rats (Farkas et al. 2004; Farkas et al. 2005; Xu et al. 2010).

In the MWM task, the escape latency time and traveled distance to reach the hidden platform during trials and the time

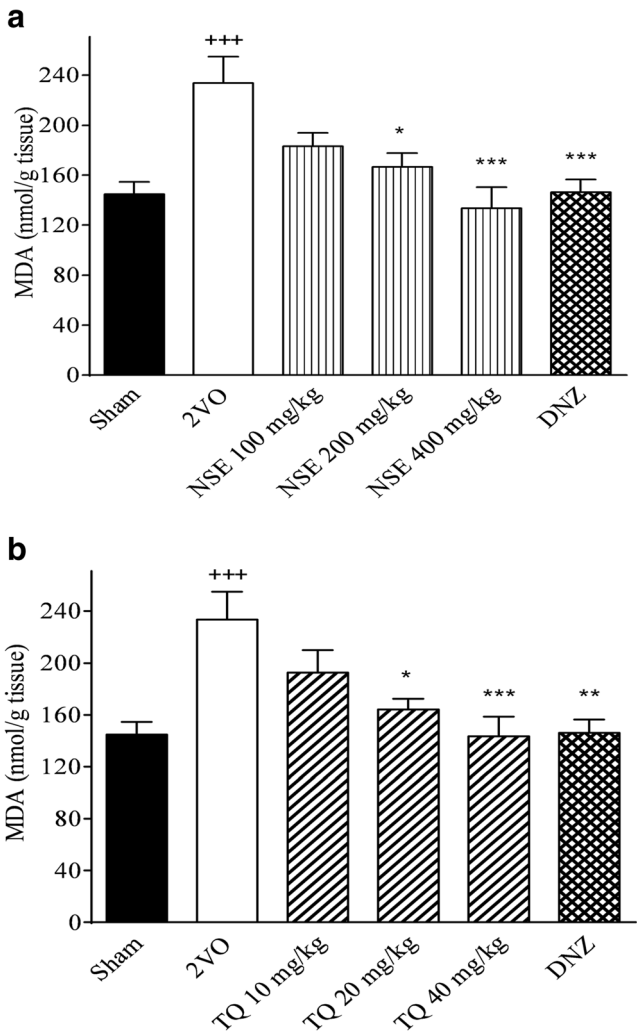

Fig. 4 Effects of *N. sativa* hydroalcoholic extract (NSE) (**a**) and thymoquinine (TQ) (**b**) on malondialdehyde (MDA) level in rats' hippocampus. Values are means ± SEM (n = 10). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. two vessels occluded (2VO) rats, $^{+++}p < 0.001$ vs. sham group

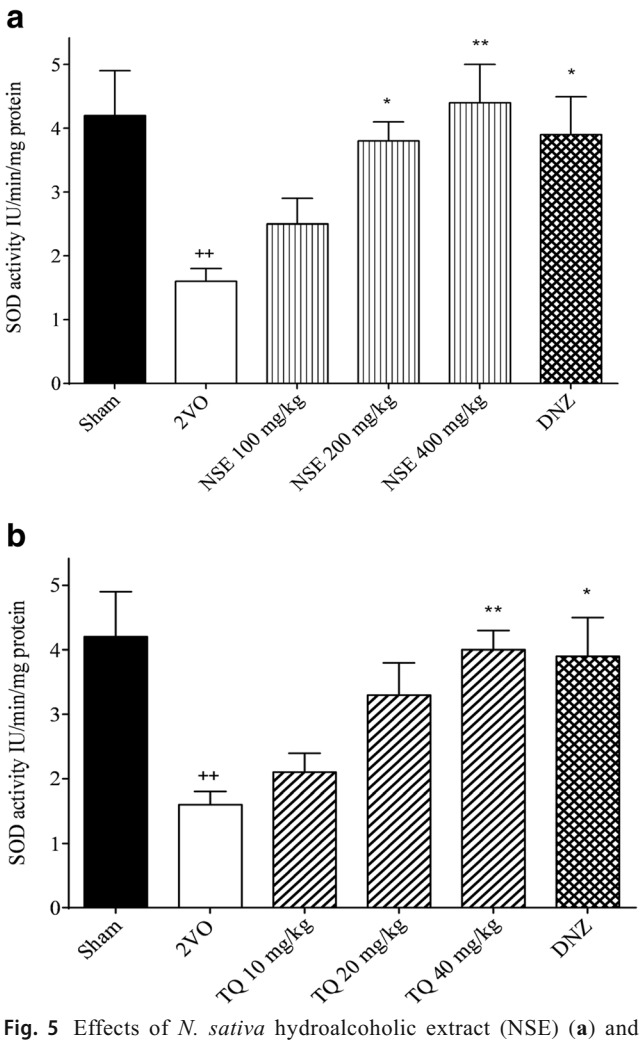

Fig. 5 Effects of *N. sativa* hydroalcoholic extract (NSE) (**a**) and thymoquinine (TQ) (**b**) on superoxide dismutase (SOD) activity in rats' hippocampus. Values are means ± SEM (n = 10). $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. two vessels occluded (2VO) rats. $^{++}p < 0.01$ vs. sham group (n = 10)

spent in the target quadrant during the probe trial were used to assess the acquisition of learning and memory in the drug-treated cerebral hypoperfused rats. The data showed that treatment with NSE, TQ, and donepezil, significantly prevented hypoperfusion-induced learning and memory deficits. The beneficial effects of *N. sativa* or its constituents on learning and memory have been also indicated in previous studies. For example, *N. sativa* oil was shown to enhance the spatial working memory performance of rats on a radial arm maze (Sahak et al. 2013), and improved spatial cognitive functions of rats with global cerebrovascular hypoperfusion (Azzubaidi et al. 2012). Furthermore, the chronic oral administration of *N. sativa* oil could enhance the consolidation and recall capability of stored information and spatial memory in diabetic animals (Jalali nodoushan and Roghani 2009). The beneficial effects of *N. sativa* on human cognition, memory, and attention have also been demonstrated (Bin Sayeed et al. 2013). *N. sativa* has been also postulated to have anxiolytic and antidepressant activities that can improve the sickness behavior induced by lipopolysaccharide in male Wistar rats (Norouzi et al. 2016).

In the present study, we showed that NSE and TQ significantly alleviated hippocampal lipid peroxidation and enhanced SOD activity following cerebral hypoperfusion. Numerous studies have indicated that oxidative stress is strongly related to the pathogenesis of cognitive impairments due to normal aging and neurodegenerative disorders such as Alzheimer's disease and vascular dementia (Clausen et al. 2012; Liu and Zhang 2012; Xie et al. 2012). Permanent cerebral hypoperfusion produces excess free radicals and weakens the antioxidant defense system, mainly SOD (Liao et al. 2004; Xu et al. 2010), thereby inducing neuronal degeneration and death (Liu et al. 2007; Xu et al. 2010). Many studies have shown that potent antioxidants and free radical scavengers

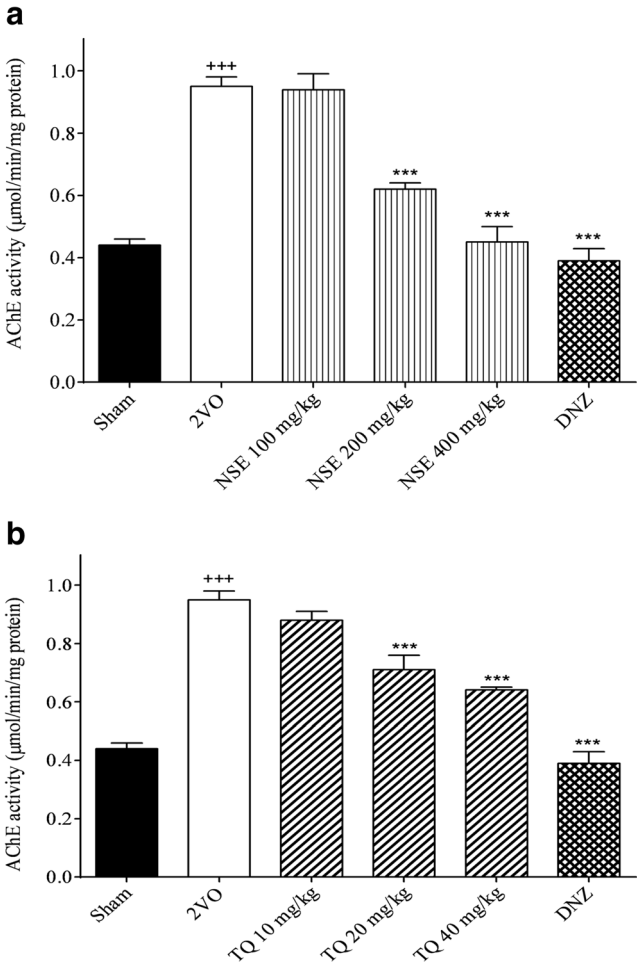

Fig. 6 Effects of *N. sativa* hydroalcoholic extract (NSE) (**a**) and thymoquinine (TQ) (**b**) on acetylcholinesterase (AChE) activity. Values are means ± SEM (n = 10). ***$p < 0.001$ vs. two vessels occluded (2VO) rats. $^{+++}p < 0.001$ vs. sham group

protect against cognitive impairments following chronic cerebral hypoperfusion. For example, edaravone and green tea polyphenols (Xu et al. 2010; Zhang et al. 2018), as well as saffron and crocin (Hosseinzadeh et al. 2012) had protective effects against cognitive impairments induced by chronic cerebral hypoperfusion via their antioxidant effects. The extract of *N. sativa* contains many compounds with antioxidant capacity such as TQ, 4-teripineol, carvacrol, and *t*-anethole (Sharieatzadeh et al. 2011). Several studies have documented the antioxidative properties of *N. sativa* (El-Far et al. 2017; Farooqui et al. 2017) and TQ in different experimental models. It has been indicated that TQ has protective effects in the control of depression, epilepsy, Parkinson's disease, Alzheimer's disease, ischemia, traumatic brain injury, anxiety, encephalomyelitis, and brain cancer (Farkhondeh et al. 2018). Mansour et al. has shown that TQ exhibits antioxidant properties via attenuating lipid peroxidation and improving SOD activity in the liver, heart, and kidney of normal mice (Mansour et al. 2002). It has been shown that *N. sativa* is an effective remedy to prevent or treat neurodegenerative diseases and cerebral ischemia because of its antioxidant capacity (Mahmoud et al. 2002). *N. sativa* and TQ scavenge free radicals, especially superoxide anions and thereby, may protect cells from oxidative stress (Badary et al. 2003; Jrah Harzallah et al. 2012). In one study, it was shown that *N. sativa* and TQ protected against chronic toluene exposure-induced hippocampal neurodegeneration (Kanter 2008). In another study, it was indicated that *N. sativa* and TQ could decrease hippocampal lipid peroxidation in global cerebral ischemia-reperfused rats (Hosseinzadeh et al. 2007).

The cholinergic system has a very important role in cognitive processes (Blake and Boccia 2018) and based on the studies exploring cholinergic changes in the experimental models of VaD, it has been documented that cholinergic deficits with increased AChE activity is closely related to the pathophysiology of cognitive impairments (Roman and Kalaria 2006; Wang et al. 2009). The cholinergic system in the brain has regulatory effects on local blood flow (Hamel 2004; Sato et al. 2001; Sato et al. 2004). It seems that the cerebral blood flow and cholinergic system are strongly related together. Therefore, the cerebral blood flow alterations, same as which is observed in VaD, may affect the cholinergic neuron of the central nervous system (Roman 2004). In a similar way, it has been discovered that the inhibition of AChE activity protects against cerebral ischemia-induced cholinergic and metabolic changes, probably via regulation of the cerebral blood flow (Sadoshima et al. 1995; Shimizu et al. 2015; Tanaka et al. 1994). In order to understand the effects of NSE and TQ on the cholinergic system following cerebral hypoperfusion, we assayed the hippocampal AChE activity. Our results showed that the AChE activity in the hippocampus of NSE- or TQ-treated rats was lower than 2VO rats, in a dose-dependent manner. The in vitro effect of TQ on the AChE activity (Jukic et al. 2007; Khan et al. 2012) and its interaction with the cholinergic system have been shown in vivo (el Tahir et al. 1993; Wienkotter et al. 2008). Furthermore, Khan et al. indicated that TQ may be considered as an antipsychotic agent and memory enhancer through decreasing the brain level of dopamine and AChE activity (Khan et al. 2014). Anticholinesterase properties of *N. sativa* have also been reported previously (Yassin 2005). Hosseini et al. showed that *N. sativa* decreased the AChE activity and oxidative stress of the rat brain in a scopolamine-induced memory impairment model (Hosseini et al. 2015). It was shown that *N. sativa* oil mimicked the donepezil effects on improved cognitive processes via AChE inhibition, reduction of lipid peroxidation and tumor necrosis factor-alpha (TNF-) levels, and enhancement of glutathione contents (Sahak et al. 2013). It has also been revealed that *N. sativa* has the ability to improve the cognitive functions via modulating several CNS neurotransmitters such as GABA, glycine, glutamate, and aspartate (El-Naggar et al. 2010).

Conclusion

The results of the present study indicate that the treatment of cerebral hypoperfused rats with *N. sativa* extract and TQ improve learning and memory processes via mitigating hippocampal oxidative stress and AChE activity. These results suggest the beneficial effects of *N. sativa* and TQ in the cerebrovascular insufficiency states, but further research is needed to identify the full potential of these agents in vascular dementia.

Compliance with ethical standards

Declaration of conflicting interests The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

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